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Crystal Structure of Glycoside Hydrolase Family 9 Endoglucanase with an N-Terminal Ig-Like Domain Isolated from Leaf-Branch Compost

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Cellulase degrades glycosidic bonds of cellulose and is widely used for industrial purposes. Ten genes encoding novel cellulases, termed LC-CelA~J, were isolated from leaf-branch compost by a metagenomic approach. Of them, LC-CelG, which is a member of glycoside hydrolase family 9 cellulases and contain an N-terminal immunoglobulin-like (Ig-like) domain, was overproduced in E. coli, purified, and characterized. LC-CelG consists of 577 amino acid residues. LC-CelG exhibited the highest activity at 70°C and pH 7.0. The crystal structure of LC-CelG was determined at 2.15Å resolution. This structure resembles those of Clostridium thermocellum Cel9A (PDB: 1CLC), and contains two Ca^{2+} and one Zn2+ ions. Two conserved residues, Q40 and D99, in the Ig-like domain form hydrogen bonds and salt bridges respectively with the catalytic domain. To analyze the role of these interactions, two single (Q40A-CelG and D99A-CelG) and one double (Q40A/D99A-CelG) mutant proteins were constructed. The deletion mutant, ΔIg-CelG, which lacks the Ig-like domain, was also constructed. The far-UV CD spectra of these mutant proteins suggest that the structure of LC-CelG is not significantly changed by these mutations and deletion. Q40A-CelG and D99A-CelG were nearly as stable as LC-CelG, whereas Q40A/D99A-CelG and ΔIg-CelG were less stable than LC-CelG by 9.2 and 12.2°C respectively. This result suggests that Q40 and D99 cooperatively contribute to the stabilization of LC-CelG and removal of the Q40- and D99-mediated interactions is the main reason why LC-CelG is destabilized by deletion of the Ig-like domain. ΔIg-CelG was inactive at any temperature examined, whereas other mutants were as active as LC-CelG at $\leq$60°C, suggesting that the Ig-like domain is required for substrate binding or to make the conformation of the active site functional.

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Molecular Characterization of the ABA-Independent Interactions between PYl10 and PP2Cs in *Oryza Sativa*

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Abscisic acid (ABA) receptors in *Arabidopsis thaliana* (AtPYLs) and in *Oryza sativa* (OsPYLs) constitute essential components in abiotic stress response pathways in plants. Inhibition of protein phosphatases type 2C (PP2Cs) by PYLs is achieved by either ABA-dependent or ABA-independent interactions between two proteins. Despite structural and biochemical characterizations of inhibition of PP2Cs by PYLs, less is known on systematic quantitative investigation of the interaction between PYLs and PP2Cs. Here, we identified OsPYL10 as a major ABA receptor, which can interact most of OsPP2Cs with different strengths depending on ABA concentration in yeast two hybridization analysis. While the interactions between homologous proteins in *Arabidopsis thaliana* are reported to be ABA-dependent, ABA exhibited no significant effect on the interactions of OsPYL10 with OsPP2Cs probed quantitatively by biolayer interferometry. Moreover, the stabilities of such interactions varied whether OsPYL10 and OsPP2Cs form a protein complex or not. Taken together, our results provide the molecular basis for understanding ABA-independent inhibition of OsPP2Cs in rice.

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Activation Volumes of Enzymes Adsorbed on Silica Particles

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The immobilization of enzymes on carrier particles is useful in many biotechnological processes. In this way, enzymes can be separated from the reaction solution by filtering and can be reused in several cycles. On the other hand, there is a series of examples of free enzymes in solution that can be activated by the application of pressure. Thus, a potential loss of enzymatic activity upon immobilization on carrier particles might be compensated by pressure. In this study, we have determined the activation volumes of two enzymes, alpha-chymotrypsin (a-CT) and horseradish peroxidase (HRP), when they are adsorbed on silica particles and free in solution. The experiments have been carried out using fluorescence assays under pressures up to 2000 bar. In all cases, activation volumes were found to depend on the applied pressure suggesting different compressions of the enzyme-substrate complex and the transition state. The volume profiles of free and adsorbed HRP are similar. For a-CT, larger activation volumes are found in the adsorbed state. However, up to about 500 bar, the enzymatic reaction of a-CT, which is adsorbed on silica particles, is characterized by a negative activation volume. This observation suggests that application of pressure might indeed be useful to enhance the activity of enzymes on carrier particles.

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The Ion Channel Function of Mouse Chromogranin B

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Chromogranins are known to play fundamentally important roles in the biogenesis, acidification and maturation as well as the regulated release of secretory granules in almost all endocrine cells. Elevated level of chromogranin A and B has been biomarkers for multiple different neuroendocrine tumors. The function of chromogranins in the regulated secretory pathway is conserved in the whole kingdom of eukaryote. Even though immuno-histochemical data and biochemical analysis have provided key evidence that these proteins are important for sorting of granule-specific cargos and for the generation of specific peptide hormones after processing and release of the granules, the exact function of the chromogranins besides being a low-affinity calcium binder and a potential interaction partner with the IP_3R remains unclear. We are using mouse chromogranin B as our model system in order to understand some of the key functions of chromogranins. We did not detect strong direct interactions between chromogranin B and the type 1 IP_3R. Instead we observed strong calcium-dependent aggregation of chromogranin B. When reconstituted in liposomes, chromogranin B formed pH and calcium-regulated conductance, and the channels appear to conduct F^- and Cl^- but not formate, acetate or citrate. Our biochemical data demonstrated that a short fragment close to the C-terminus of the protein is the only one capable of membrane binding. Our working model is that chromogranin B not only induces biogenesis of secretory granules, but its anionic conductance functions to support the continued acidification of the luminal side of the granules, which is critical for the proper processing of the cargos inside.

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Cytoplasmic Freezing in S. Pombe upon Glucose Starvation

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Many cells and organisms react to depletion of nutrients with a low-energy program. Yeast cells response to nutrient starvation by entering a quiescent state. This state is defined by a substantial increase in the viscosity of the cell cytoplasm, a phenomenon we term ''cell-freezing'', or cytoplasmic freezing. Cell-freezing is a phenomenon that has been discovered recently through measurements of the viscoelastic properties of the cytoplasm of fission yeast cells. It occurs as a response to carbon starvation and leads to a dramatic immobilization of all visible sub-cellular structures, which suggests that the preservation of the internal organization of the cell in this quiescent state is its main biological function.

Recently we found evidence that the transition of viscosity of the cell cytoplasm could be reliably reproduced by starving cells of glucose. Also, there is evidence that septins might be involved. Septins were known to form fibers in vitro, and during cell division. Hence, we proposed that a dense and homogeneous network will be formed by septins when the cell enters the starvation state and that this network is sufficiently sturdy to immobilize all major cell components.

We used conventional and Cryo-Electron Microscopy (cryo-EM) combined with tomographic 3-D analysis, that allowed us visualizing polymeric structures resembling that of septin filaments, and their interaction with subcellular structures. In particular, high pressure freezing followed by vitrified sectioning (Cryo-microtome) technology preserved the original condition of cell to molecular detail. Here we display for the first time the proposed filamentous network in starved fission yeast cells. Apart from filament bundles we also found some other interesting novel sub-cellular structures.